Background Systemic lupus erythematosus (SLE) is a multifactorial autoimmune disease, in which infections are considered to play a pathogenic role, but the cellular and molecular culprits remain unknown. Bacterial biofilms are multicellular bacterial communities important in the establishment of chronic infection by pathogens. Bacteria produce amyloids, complex proteins with a conserved beta sheet structure, that strengthen the extracellular matrix of their biofilms. We have reported that bacterial and eukaryotic DNA is incorporated into curli fibres, functional bacterial amyloids present in Salmonella and E. coli biofilms. We found that curli/DNA complexes from biofilms activate conventional dendritic cells leading to production of pro-inflammatory cytokines, including Type I interferons, in vitro and in vivo, indicating that curli are a new class of danger signals. I.p. infections with curli-expressing Salmonella enterica serovar Typhimurium or E. Coli Nissle, and also curli-deficient Salmonella enterica serovar Typhimurium and E. Coli Nissle, in vivo infections, bone marrow-derived cultures of conventional dendritic cells and macrophages were generated in vitro and B cells were isolated ex vivo from NZBxW/F1 lupus-prone mice, TLR-deficient mice and wild-type mice and stimulated with curli/DNA complexes purified from Salmonella biofilms. Moreover, lupus prone and control mice were infected i.p. and by oral gavage with curli-expressing or curli-deficient S. Typhimurium and the effects on dendritic cells, macrophages, and B cell activation, and production of autoantibodies were studied. A novel ELISA was developed to measure anti-curli antibodies in SLE patients and infected mice.

Materials and methods In order to determine the cellular and molecular players in the acceleration of lupus by curli-expressing infections, bone marrow-derived cultures of conventional dendritic cells and macrophages were generated in vitro and B cells were isolated ex vivo from NZBxW/F1 lupus-prone mice, TLR-deficient mice and wild-type mice and stimulated with curli/DNA complexes purified from Salmonella biofilms. Moreover, lupus prone and control mice were infected i.p. and by oral gavage with curli-expressing or curli-deficient S. Typhimurium and the effects on dendritic cells, macrophages, and B cell activation, and production of autoantibodies were studied. A novel ELISA was developed to measure anti-curli antibodies in SLE patients and infected mice.

Results We found that Curli and DNA synergistically activate innate and adaptive immune cells, in vitro and in vivo, via TLR2 and TLR9, leading to a Type I interferon response. We found that curli/DNA complexes can directly stimulate proliferation and...
activation of B cells and induce autoantibody production. Natural infections with curli-expressing S. Typhimurium triggered autoantibodies production in NZBxW/F1 mice and control mice, suggesting curli/DNA complexes break tolerance in SLE. Finally, sera from lupus patients during flares showed elevated levels of anti-curli antibodies.

**Conclusions** Biofilm-derived curli/DNA complexes are potent activators of innate and adaptive immune cells and can mediate the acceleration of lupus by infections. These studies may provide a novel biomarker of flare and suggest targeting biofilms and bacterial infections as new therapeutic tools in lupus.

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**Materials and methods** T cell epitope mapping was done by the generation and characterisation of TT hybridomas to SmD and Ro60 and their 15 mers. Antibody specificities to SLE-related Ags were calculated. 18 of reactive T-T hybridomas had no sequence homology. 18 of reactive T-T hybridomas

**Results** At least seven SmD core T cell epitopes were identified. They have no sequence homology. 18 of reactive T-T hybridomas used multiple TCRα and TCRβ. Bioinformatics analysis identified more than 10000 potential bacterial mimic peptides with many from commensal bacteria. The binding affinities of the SmD T epitopes were in the medium range among all the relevant mimics. Selected mimic peptides showed that only those with medium binding affinity were related to the SmD peptides and only they were able to stimulate Ab responses in patterns similar to that induced by the related SmD peptide. A significant number of TT hybridomas were reactive with multiple T cell epitopes within the SmD molecules. Some of these hybridomas also were reactive with SmD, SmB and/or A protein within the snRNP particles and Ro60. In addition, the bacterial T cell mimic peptides often shared B cell epitopes with the related SmD peptide. Also healthy DR3+ blood donors had significantly higher Ab titers against SmD. Similar results were obtained in the Ro60/La system.

**Conclusions** Autoactive TCRs are part of the normal repertoire that are positively selected for host defense against microbial agents. The presence of multiple intra- and inter-molecular T and B cell epitopes are characteristic of SLE-related auto-Ags. These characteristics provide a scenario for the inevitability of the presence of auto-Ab and autoactive-T cells in healthy individuals with susceptible HLA-D regions and provide a mechanism for B cell epitope spreading in SLE. SLE-related Abs are the results of our reaction to exposure to commensal and/or pathogenic microbes. Innate immunity plays an amplifying role. These observations provide the rationale to target microbiome and both adaptive and innate immunological responses in the treatment of SLE.

**Abstracts**

**AI-28**

**HLA-DR3 RESTRICTED RESPONSES TO SMD, A LUPUS-RELATED ANTIGEN PROVIDE INSIGHTS TO THE ORIGIN OF LUPUS-RELATED AUTOANTIBODIES AND THE UNIQUE FEATURES OF THE TARGETED ANTIGENS**

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**Background** The presence of complex autoantibodies (auto-Abs) is a hallmark of SLE. The most cited hypotheses for their origin are the “B cell epitope mimicry hypothesis between SmB and EBV” and the “particle hypothesis”. Neither is adequate to explain the characteristics of SLE-related Abs. Our studies showed that HLA-DR3 and DR2 transgenic mice in contrast to DR4 respond well to SmD and Ro60 with DR3 mice being the best responders. In addition, certain bacterial mimics of SmD and Ro60 T cell epitopes were shown to induce auto-Abs to SmD and Ro60/La.

**Materials and methods** T cell epitope mapping was done by the generation and characterisation of TT hybridomas to SmD and Ro60 and their 15 mers. Antibody specificities to SLE-related antigens (Ags) and their peptides were done by ELISA.

**Results** At least seven SmD core T cell epitopes were identified. They have no sequence homology. 18 of reactive T-T hybridomas used multiple TCRα and TCRβ. Bioinformatics analysis identified more than 10000 potential bacterial mimic peptides with many from commensal bacteria. The binding affinities of the SmD T epitopes were in the medium range among all the relevant mimics. Selected mimic peptides showed that only those with medium binding affinity were related to the SmD peptides and only they were able to stimulate Ab responses in patterns similar to that induced by the related SmD peptide. A significant number of TT hybridomas were reactive with multiple T cell epitopes within the SmD molecules. Some of these hybridomas also were reactive with SmD, SmB and/or A protein within the snRNP particles and Ro60. In addition, the bacterial T cell mimic peptides often shared B cell epitopes with the related SmD peptide. Also healthy DR3+ blood donors had significantly higher Ab titers against SmD. Similar results were obtained in the Ro60/La system.

**Conclusions** Autoactive TCRs are part of the normal repertoire that are positively selected for host defense against microbial agents. The presence of multiple intra- and inter-molecular T and B cell epitopes are characteristic of SLE-related auto-Ags. These characteristics provide a scenario for the inevitability of the presence of auto-Ab and autoactive-T cells in healthy individuals with susceptible HLA-D regions and provide a mechanism for B cell epitope spreading in SLE. SLE-related Abs are the results of our reaction to exposure to commensal and/or pathogenic microbes. Innate immunity plays an amplifying role. These observations provide the rationale to target microbiome and both adaptive and innate immunological responses in the treatment of SLE.

**AI-29**

**METABOLOME CHECKPOINTS OF MTOR ACTIVATION AND CLINICAL RESPONSIVENESS IN SLE**


**Background** The mechanistic target of rapamycin (mTOR) serves as a metabolic sensor of genetic and environmental cues that effectively regulates physiological T-cell activation and lineage specification. mTOR complex 1 (mTORC1) promotes pro-inflammatory T-cell development, B-cell activation and production of antinuclear autoantibodies (ANA) both in patients and mice with systemic lupus erythematosus (SLE). Therefore, we initiated a prospective clinical trials with rapamycin and N-acetyl-cysteine (NAC), the latter of which blocks redox-dependent mTORC1 activation.

**Materials and methods** 36 SLE patients were enrolled in a 3-month placebo-controlled trial with NAC which involved 212 metabolic and immunological markers prior to enrollment, and follow-up at 1-month intervals. 42 healthy controls, matched at each visit for age, ethnicity, and gender, were studied in parallel. 258 metabolites were measured mass spectroscopy. 40 SLE patients were enrolled in a 12-month open-label intervention with rapamycin; patients and 88 matched controls were studied in parallel at 3-month intervals. Analysis of pathways, area under the curve (AUC) logistic regression, two-factor (NAC versus placebo) time series within individual subjects were performed with Metaboanalyst. SLEDAI, BILAG, and SRI disease activity indices were calculated.

**Results** Rapamycin reduced disease activity in 126 ± 18 days as evidenced by well-tolerated rapamycin plasma levels of 8.7 ± 1.2 ng/ml, which was within the targeted therapeutic range of 6–15 ng/ml. SLEDAI disease activity scores were reduced to 5.7 ± 1.0 from 11.8 ± 1.1 at baseline (p = 0.0028). Among the patients who completed the 1 year intervention, a SLE Responder Index of 64.3% was achieved. Rapamycin inhibited the pro-inflammatory T cell skewing (Figure 1), including the expansion and IL-4 production of CD4+CD8+ double-negative (DN) T cells and reversed the contraction of CD4+CD25+FoxP3+ regulatory T cells (Tregs) and CD4+ and CD8+ central and effector memory T cells. Treatment with NAC exerted lesser but statistically significant reduction of SLEDAI and BILAG over 3-month. Metabolome changes involved 27 of 80 KEGG pathways at FDR p < 0.05 with most prominent impact on the pentose phosphate pathway (PPP). While cytochrome was depleted, a PPP-regulated compound, kynurenine, was the most increased metabolite and the top predictor of SLE (AUC = 0.859). Kynurenine directly stimulates mTORC1 activity of DN T cells in vitro. Relative to placebo, NAC reversed these metabolite changes in vivo.

**Conclusions** The PPP-connected accumulation of kynurenine and its stimulation of mTORC1 are identified as metabolic pathways.